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Zana H. Alzangana\*  
Ahmed T. Taha

## Effect Quercetin on Physiological and Productive Performance of females quail exposed to oxidative stress induced by H<sub>2</sub>O<sub>2</sub>

### ABSTRACT

Plant polyphenols, particularly flavonoids, are of great intrigued due to their wide run of natural activities. Quercetin, an omnipresent flavonoid, is known to have antioxidant and antibacterial impacts. The aim of this experiment is to know the effect of adding different levels of quercetin on some productive and physiological Traits of normal and oxidative stress quail birds. In the study, (108) females quail at the (45 days)of age, randomly distributed into six treatments, with three replications per each and reared for (42 days). The experiment treatments, as follows: The first treatment (T1): control group and the second treatment (T2): the birds fed a standard diet and drinking water with hydrogen peroxide (0.01% H<sub>2</sub>O<sub>2</sub>), the third(T3) and fourth(T4) treatments :The birds fed a standard diet with (400 and 600 mg of Quercetin / kg feed) respectively, normal drinking water. About the fifth (T5) and six (T6) treatments,the birds consumed same as T3 and T4 diet except for supplemented drinking water with (0.01% H<sub>2</sub>O<sub>2</sub>).The results indicated that the addition of quercetin to diets of quail birds led to:Significant improvement in the production Traits of natural and stressful quails by increasing egg production rate. Significant improvement of some biochemical indicators in the blood serum of natural and stressful quails birds, such as Estrogen, cholesterol, total protein, globulin, Calcium, AST, ALT, Malondialdehyde enzyme and glutathione concentration. While it did not affect glucose and albumin concentration. In conclusion, our study present that quercetin has huge effect on production and physiological indicators in females quail.

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Department of Animal  
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## INTRODUCTION

Current inclinations aim to use feed additives in the development of the poultry industry by improving growth performance, feed conversion ratio and health status through biochemical indicators (Liu et al., 2014). As well as increasing egg production and improving reproductive indicators by antioxidant properties (Yang et al., 2018). Oxidative stress is the state of an imbalance between the production of reactive oxygen species (Free radicals) and the ability of biological systems to eliminate or correct their negative effects (Turko et al. 2001; Maritim et al. 2003). Free radicals have a high ability to damage or destroy vital molecules in different body cells such as lipids, proteins, nucleic acids and carbohydrates, and that the damage caused by free radicals has a great role in increasing the generation of these free radicals continuously and through a series of reactions that contribute to the breakdown of vital molecules in the cell (Cheng et al., 2002). Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called “free-radical scavengers.” The sources of antioxidants can be natural or artificial (Lee and Lim, 2006). Antioxidants in the body eliminate free radicals that are constantly

\* Corresponding author: E-mail: [zanahasan88@yahoo.com](mailto:zanahasan88@yahoo.com)

emerging as a result of vital processes in the body, Antioxidants also prevent the generation of free radicals and oxidative processes in the body, Antioxidants reduce the risk of oxidative damage and infection with a number of diseases. As a result, antioxidants are considered to have a defensive role against the catabolic activity of free radicals in terms of the principle of their generation and the chain of harmful interaction (Prakash and Joshi 2004). The greatest advantage of quercetin as an antioxidant is its ability to search and scavenge for free radicals (ROS) that can cause various diseases such as diabetes, cancer, and chronic infections (Alrwaiq& Abdullah, 2014). Regardless of the importance of quercetin as an excellent antioxidant, it has anti-inflammatory and immunomodulatory properties in a special way (Serafani et al., 2010). Supplemental quercetin to poultry diet is important because it improves production performance and has positive effects on blood parameters (Kim et al., 2015). The aim of the present study is to investigate the effect of different levels of quercetin in production and physiological performance of quail birds raised under conditions of oxidative stress.

## MATERIAL AND METHODS

### The Experiment design:

A total of, (108) females at (45) days of age. Raised for (42 days) in 18 the floor cages, reared in each six females. The experiment included six treatments according to the following:

The 1sttreatment (T1): the control group the birds were fed on a standard diet and natural drinking water.

The 2ndtreatment (T2): The birds fed on a standard diet and hydrogen peroxide( H<sub>2</sub>O<sub>2</sub>) at a concentration of 0.01% in drinking water.

The 3rdtreatment (T3): The birds fed on a standard diet + (400 mg of quercetin / kg diet) and natural drinking water.

The 4thtreatment (T4): The birds fed on a standard diet + (600 mg quercetin / kg diet) and natural drinking water.

The 5thtreatment (T5): The birds fed on a standard diet + (400 mg quercetin / kg diet) + (0.01%) H<sub>2</sub>O<sub>2</sub> in drinking water.

The 6th treatment (T6): The birds fed on a standard diet + (600 mg quercetin / kg diet) + (0.01%) H<sub>2</sub>O<sub>2</sub> in drinking water.

Feed and water provide to the birds ad libitum.

### Hen-day egg production (H.D%)

Egg were daily collected at 12:30 pm and recorded as follows(Al-Zubaidy,1986):

$$\% \text{ H.D} = \frac{\text{Total number of eggs}}{\text{Number of live hens} \times 42 \text{ days}} \times 100$$

### Physiological indicators of blood tests:

Blood samples collected at the end of the study period by Euthanasia method. The blood was collected in special tubes. After blood to coagulate Placed in a centrifuge of 3000 rpm / 20 minutes, to obtain blood serum and kept at freezing temperature (-20oC) until analyses. Glucose, Cholesterol, concentrations, AST and ALT activity and estrogen hormone concentrations in blood serum were determined by using test kits. Glutathione (GSH) was measuring by method of (Kamencic et al, 2000), malondialdehyde (MDA) was estimated as method of (Guidet and Shah,1989).

### Statistical Analysis:

Statistical data analysis was performed using Completely Randomized Design to measure the effect of the treatments on the studied characteristics in this experiment. Using the Duncan's Multiple Range Test (Duncan, 1955), the significant differences between the averages estimated. The statistical program (SAS, 2005) .

## RESULT

In the current experiment, egg production (H.D%) showed significantly reduction in T2 stressful group. Treatment with quercetin T3 and T4 lead to improvement in egg production as compared with T2, T5 and T6. Nevertheless, no significant differences between T5 and T6 compared with

control group . As shown in the figure (1).It is evident from the results shown in figure(2)the effect of adding different concentrations of quercetin to the diets of female quail birds, as the concentration of the estrogen hormone increased in the T3 and T4 compared to the T2, although it did not differ with the control group . However, we notice that there is no significant difference between T5 and T6 with control group and T2.

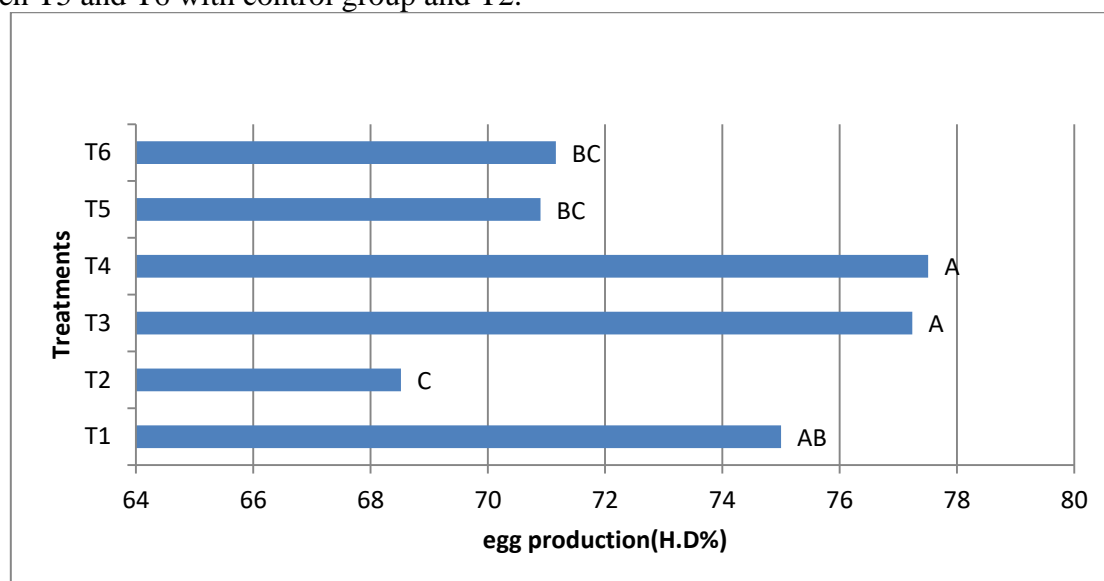


Figure (1) Effect of adding different levels of quercetin on egg production (H.D%) of females quails exposed to oxidative stress induced by  $H_2O_2$

\* Means having different letters in the same rows are significantly different at  $P < 0.05$

T1: reserved standard diet and normal water. T2: reserved standard diet and (0.01%) hydrogen peroxide in drinking water. T3: feed supplemented by (400 mg quercetin / kg diet) with normal water. T4: feed supplemented by (600 mg quercetin / kg diet) with normal water. T5: feed supplemented by (400 mg quercetin / kg diet) with (0.01%)  $H_2O_2$  in drinking water. T6: feed supplemented by (600 mg quercetin / kg diet) with (0.01%)  $H_2O_2$  in drinking water.

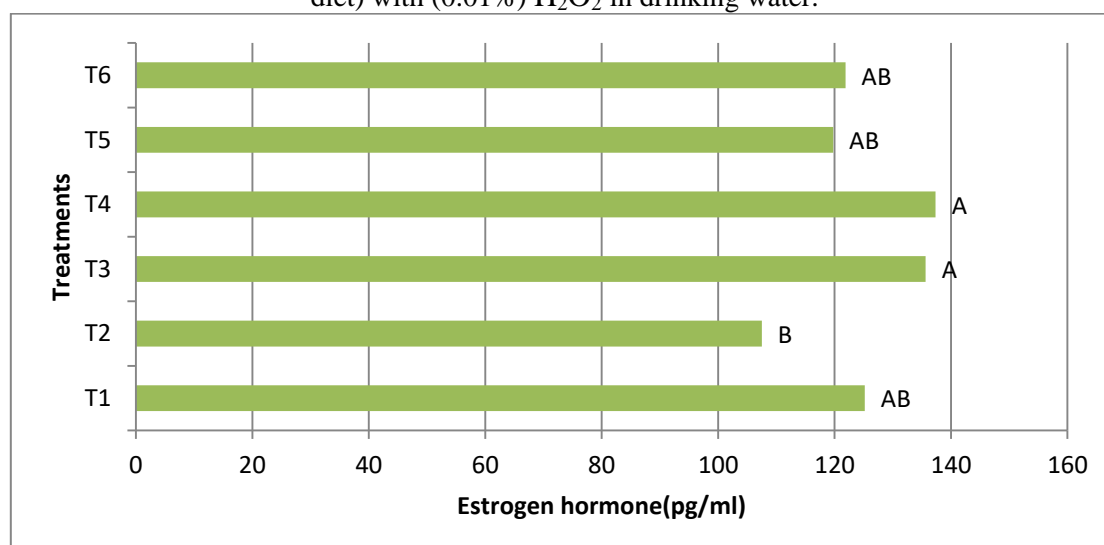


figure (2) Effect of adding different levels of quercetin on estrogen hormone concentration of females quails exposed to oxidative stress induced by  $H_2O_2$

\* Means having different letters in the same rows are significantly different at  $P < 0.05$ .

T1: reserved standard diet and normal water. T2: reserved standard diet and (0.01%) hydrogen peroxide in drinking water. T3: feed supplemented by (400 mg quercetin / kg diet) with normal water. T4: feed supplemented by (600 mg quercetin / kg diet) with normal water. T5: feed supplemented by (400 mg quercetin / kg diet) with (0.01%)  $H_2O_2$  in drinking water. T6: feed supplemented by (600 mg quercetin / kg diet) with (0.01%)  $H_2O_2$  in drinking water.

The results shown in table (1), indicate that there were no significant differences in glucose concentrations among all treatments adding quercetin to normal and stressful birds compared with the control group and T2. The cholesterol concentration was not affected when comparing the treatment of addition of quercetin for natural and stressed birds with the T4 which achieved a significant decrease compared with T2. Total protein and albumin not affected with adding different level of quercetin as compared with control group and T2, whereas T4 recorded significantly increasing in total protein concentration comparison with other treatments expected T3.

Adding quercetin at level 600 mg/kg diet T4 leads to increasing in globulin concentration compared with control group and both treatments 5 and 6.

Calcium concentration significantly reduced with H<sub>2</sub>O<sub>2</sub> adding to drinking water in quail birds compared with control group and treatments. The treatment by quercetin leads to led to a significant decrease in the level of serum AST, ALT, as compared with the T2 group (P≤0.05). While no significant differences between all treatments and control group. Supplemented quail birds with H<sub>2</sub>O<sub>2</sub> leads to significantly increasing in malondialdehyde (MDA) concentration compares with other treatments, quercetin supplemented to the normal and stressful birds caused improvement in antioxidant parameters by reduction MDA levels and increasing glutathione (GSH) concentration as comparison to control group or T2 group.

**Table (1) effect of adding different levels of quercetin on some biochemical characteristics of females quails exposed to oxidative stress induced by H<sub>2</sub>O<sub>2</sub>**

Treatment	T1	T2	T3	T4	T5	T6
Treats						
Glucose (mg/dl)	154.53± 2.52 A	145.71± 10.30 A	151.59± 12.15 A	148.73± 12.92 A	165.55± 3.18 A	163.12± 6.61 A
Cholesterol (mg/dl)	245.00± 3.78 AB	253.00± 2.64 A	242.33± 6.38 AB	235.33± 4.40 B	245.00± 6.42 AB	246.00± 5.29 AB
Total protein (g/dl)	3.80 ± 0.23 B	4.10± 0.05 B	4.33± 0.12 AB	4.70 ± 0.11 A	3.96± 0.31 B	3.83± 0.08 B
Albumin (g/dl)	1.42± 0.17 A	1.18± 0.06 A	1.25± 0.08 A	1.36± 0.08 A	1.36± 0.02 A	1.35± 0.02 A
Globulin (g/dl)	2.38 ± 0.06 D	2.92± 0.01 ABC	3.08± 0.19 AB	3.33± 0.08 A	2.60± 0.31 BCD	2.47± 0.06 CD
Calcium (mg/dl)	24.08± 0.68 A	18.37± 0.85 B	24.19± 3.02 A	26.34± 1.36 A	22.33± 1.02 AB	21.41± 1.22 AB
ALT (U/l)	31.33± 1.76 ABC	37.00± 2.08 A	29.66± 1.76 BC	26.33± 1.76 C	34.33± 2.02 AB	32.66± 1.45 AB
AST (U/l)	105.00± 5.19 BC	128.00± 1.73 A	104.66± 3.71 BC	93.33± 6.17 C	117.66± 3.17 AB	105.00± 2.64 BC
MDA(μmole/mole)	1.36± 0.09 CD	1.98± 0.06 A	1.28± 0.02 D	1.43± 0.12BCD	1.63± 0.04 B	1.56± 0.05 CB
Glutathione (μmole/mole)	2.62± 0.17 BCD	2.23± 0.06 D	2.98± 0.11 AB	3.09± 0.11 A	2.41± 0.15 CD	2.73± 0.13 ABC

\* Means having different letters in the same rows are significantly different at P<0.05.

T1: reserved standard diet and normal water. T2: reserved standard diet and (0.01%) hydrogen peroxide in drinking water. T3: feed supplemented by (400 mg quercetin / kg diet) with normal water. T4: feed supplemented by (600 mg quercetin / kg diet) with normal water. T5: feed supplemented by (400 mg quercetin / kg diet) with (0.01%) H<sub>2</sub>O<sub>2</sub> in drinking water. T6: feed supplemented by (600 mg quercetin / kg diet) with (0.01%) H<sub>2</sub>O<sub>2</sub> in drinking water.

## DISCUSSION

The significant improvement in egg production when added quercetin to the quail diets corresponds to the results of Liu et al. (2014), which found a correlation between the rate of egg production and the addition of quercetin to the diets of laying hens. It also agreed with Yang et al (2018), where they obtained an increase in the rate of egg production with the addition of different levels of quercetin without a change in egg weight. It disagreed with Iskender et al. (2017), who found no significant differences in the rate of egg production of laying hens treated with quercetin. This improvement may be due to several possible reasons, including the nutritional aspect and the mechanism of utilizing nutrients, which indicates the importance of quercetin in improving the actions of food digestion and utilization, as Liu et al. (2014) noted a significant improvement in the microbiology of hens treated with quercetin. Another aspect may be the action of quercetin through its positive effect on reproductive hormones, as we note from the results of this study a significant improvement in estrogen levels, and this may be the reason for the increase in egg production and the improvement of productive characteristics. The production of eggs is controlled by axis the hypothalamic-pituitary - The ovary, where the hypothalamus works to release GnRH to stimulate the frontal lobe of the pituitary gland to secrete the hormones FSH and LH to stimulate granule cells in the ovary to secrete the hormone estrogen, this hormone has a major role in stimulating the formation of eggs as well as its role in regulating the secretion of sex hormones from Hypothalamus and pituitary and these hormones were increased by quercetin (Yang et al., 2018).

The high levels of ALT and AST enzymes in stressed group of birds may reflect the state of cell damage, especially the hepatic ones (Al-Daraji et al., 2008). As indicated that oxidative stress increases the cases of damage to body cell membranes due to their high content of long-chain polyunsaturated fatty acids (PUFA), which causes a defect in the facultative permeability trait and this leads to penetration of these enzymes into the outside of the cell and their content in the blood serum (Li et al., 2015). Perhaps the significant increase in the levels of MDA our current study reflects the reality of this perception, as this compound is considered an important indicator of cases of oxidative damage. The improvement in the birds of the third, fourth, fifth, and sixth treatments can be attributed to the effect of quercetin, which worked beneficial to the unique combination of scavenging free radicals or cutting their interactions chains (Ying et al. 2020). the trustful confirms of this assumption is the significant improvement in glutathione (GSH) levels in our current study by addition of quercetin.

Hydrogen peroxide can affect by initiating a chain reaction leading to oxidative stress by increasing the production of oxygen in the stomach, which in turn enters the blood and leads to hyperoxia in the tissues leading to an increase in the production of ROS, H<sub>2</sub>O<sub>2</sub> associated with oxidative metabolism (Loven and Oberly, 1985). Perhaps the explanation for the decrease in GSH concentration is due to the increased oxidation of glutathione GSH to the oxidative form of disulfur (GSSG), a process induced by the enzyme GSH-PX and that the protection that glutathione peroxidase provides to cells and tissues against lipid peroxidation depends on Re-converting oxidized glutathione (GSSG) to reduced concentration of glutathione (GSH) (Little and O'Brien, 1968). This process depends on another enzyme (GSH-RD), which needs the pentose shunt activity, to provides it with the enzyme accompaniment NADPH, which works with the GSH-RD enzyme in order to re-reduce the oxidized form GSSG to the effective reduced form GSH, and that the concentration decreases GSH in oxidative stress states occurs due to a decrease in the activity of the pentose shunt in these cases. The activity of the enzyme G-6-PDH is necessary for the activity of the pentose shunt activity when the enzyme decreases due to the formation of NADPH decreases and the GSH-RD is not able to re-reduce the oxidized form of GSSG and decrease in the reduced concentration of GSH. The decrease in GSH as a result of treatment with H<sub>2</sub>O<sub>2</sub> was associated with

a significant increase in the MDA concentration in the same table as a result of high lipid peroxidation, which increases due to an imbalance between antioxidants and oxidants (Martins et al., 1985). Macdonald et al., (2010) have previously noted the existence of a significant negative correlation coefficient between GSH concentration and MDA concentration. This promotes the previous explanation and confirms the process of dependent on GSH concentration as an indicator of oxidative stress states.

The role of quercetin in lowering cholesterol was associated with inhibiting the activity of the enzyme HMG-CoA reductase, which is the first step enzyme in the cholesterol synthesis process (Lee et al., 2003), or a cholesterol metabolism regulator (Zhao et al., 2011). quercetin has the ability to regulate lipid metabolism and catabolism, preventing gene expression in broiler adipocytes (Li et al., 2013) and lipogenesis via cAMP signaling pathways (Ouyang et al., 2013), thus reducing deposition of Fat in broilers. Fats are mainly made in chicken liver, where the fats are in the form of cholesterol, phospholipids and triglycerides, and the cholesterol content is the main indicator of lipid metabolism. High levels of lipids lead to the accumulation of cholesterol, which in turn leads to rise the fatty liver disease (Quesada et al., 2009). Cholesterol is the key factor of the fatty acid oxidation process, which catalyzes the first step in the oxidative reaction of the FA peroxidase, An increase in the expression of cholesterol in mitochondria primarily promotes fatty acid oxidation (Bonfont et al., 2004).

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تأثير الكرستين على الأداء الفسيولوجي والإنتاجي لإناث طيور السمان المعرضة للإجهاد التأكسدي المستحدث باستخدام  $H_2O_2$

زانا حسن الزنكنة أحمد طاييس طه

قسم الانتاج الحيواني / كلية الزراعة / جامعة تكريت / العراق

### الخلاصة

يعتبر الكرستين واحدا من المواد الفلافونيدية الواسعة الانتشار وتعتبر النباتات المصدر الأساسي لإنتاجه. له تأثيرات مضادة للأكسدة ومضادة للبكتيريا. الهدف من هذه التجربة هو معرفة تأثير إضافة مستويات مختلفة من الكيرستين في بعض الصفات الإنتاجية والفسيولوجية لطيور السمان الطبيعية المجهدة تأكسديا. في هذه الدراسة، تم توزيع (108) إناث السمان بعمر (45 يوماً) بشكل عشوائي على ستة معاملات، بمعدل ثلاثة مكررات لكل معاملة وربيت لمدة 42 يوماً. وكانت معاملات التجربة على النحو التالي: المعاملة الأولى (T1): مجموعة السيطرة. المعاملة الثانية (T2): غذيت الطيور على عليقة قياسية وماء شرب اضعف له بيروكسيد الهيدروجين بنسبة ( $H_2O_2$  0.01 %). المعاملتين T3 وT4: الطيور غذيت على عليقة قياسية مع (400 و 600 ملغم الكيرستين / كغم العلف) على التوالي، وماء شرب اعتيادي. أما في المعاملتين T5 وT6: غذيت الطيور على نفس نسبة T3 و T4 فيما عدا ماء الشرب اضعف له ( $H_2O_2$  0.01 %)، وأظهرت النتائج أن إضافة الكرستين إلى علائق طيور السمان أدى إلى: تحسن معنوي في الصفات الإنتاجية لطيور السمان غير المجهدة والمرباة تحت ظروف الإجهاد التأكسدي، عن طريق زيادة معدل انتاج البيض وتحسن معنوي في بعض المؤشرات الكيموحيوية مثل تركيز هرمون الاستروجين والكوليسترول وتركيز البروتين الكلي والكلوبيولين و الكالسيوم و إنزيم AST و ALT، وتركيز المألون ثنائي الديهايد وتركيز الكلوتاتيون. بينما لم يؤثر معنوياً على تركيز الكلوكوز وتركيز الألبومين. تتبين من دراستنا ان للكرستين تأثير عظيم على الصفات الفسلجية والصفات الإنتاجية في إناث طيور السمان.

### الكلمات المفتاحية:

الكرستين، السمان، انتاج البيض، صفات الدم، مضادات الأكسدة.